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Exploratory study on the endogenous ouabain in idiopathic pulmonary arterial hypertension patients

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Abstract

Introduction. Endogenous ouabain (EO) is a steroid hormone secreted by the adrenal glands associated with adverse cardiovascular outcomes. However, EO plays other roles as brain protection against traumatic injury and seems involved in the adaptive response to hypoxia. Recently, we detected, for the first time, EO in a healthy human group of acute hypoxia and diving animals.

Methods. This study complements the above as we considered a human model of chronic hypoxia. The aim is to detect EO in five idiopathic pulmonary arterial hypertension patients.

Results and Discussion. We found that these patients had higher plasma concentrations of EO than control subjects. In addition, EO plasma concentrations were negatively correlated with the mean pulmonary arterial pressure and total pulmonary vascular resistance. The results could suggest that high concentrations of EO are predictive of better adaptation of the right ventricular afterload.

Conclusion. Although the results are preliminary, they can represent a helpful hint for future investigations for possible therapeutic and diagnostic approaches.

Key words

- hypoxia
- endogenous ouabain
- pulmonary arterial hypertension
- chronic disease

INTRODUCTION

Acute and chronic exposure to hypoxia reveals a range of cognitive and behavioral deficiencies [1]. However, possible explanations for the intact cognitive functioning found in mountaineers, in some clinical conditions, or apnea divers include neuroprotective factors [2] and adaptive response to low-oxygen states [3]. Among the neuroprotective factors, the endogenous ouabain (EO), a stress-related hormone secreted by the adrenal glands [4], seems to be involved in the adaptive response to hypoxia. EO is a cardiac glycoside structurally similar to digoxin [5]. Digoxin-like immunoreactive substances have been found in sleep obstructive apnea syndrome patients [6].

In Manfrini, *et al.* [7], we detected, for the first time, plasma EO in a healthy human model of acute hypoxia (18 elite apnea divers) and 31 diving animals (common bottlenose dolphins, phocids and otariids, and loggerhead sea turtles) which perform many short or long apneas without reporting neurological damages.

Human pulmonary arterial hypertension (PAH) is a pathophysiological disorder that may involve multiple clinical conditions and can complicate most cardiovascular and respiratory diseases. This disorder is characterized by right-heart catheterization showing precapillary pulmonary hypertension with a mean pulmonary artery pressure (mPAP) of >25 mmHg and a normal pulmonary artery wedge pressure of <15 mmHg [8, 9]. The classification of pulmonary hypertension (PH) has undergone a series of changes since the first classification proposed in 1973 designated only two categories: primary and secondary PH, which depend on the presence or absence of identifiable causes or risk factors [10, 11]. According to the clinical classification of PH from

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Table 1
Variables collected in IPAH (idiopathic pulmonary arterial hypertension) patients

N	Code	Age (yrs)	H (m)	W (kg)	EO (pM)	BSA (m^2)	6MWT (m)	SO_2 (%)	PAPm (mmHg)	CF (bpm)	CI ($l/min/m^2$)	PVRtot (WU)
1	IPAH40	46	1.76	101	396	2.17	590	97.0	28	85	2.40	5.18
2	IPAH41	31	1.78	55	264	1.68	410	90.0	94	90	2.20	25.26
3	IPAH42	78	1.60	80	312	1.83	300	88.0	40	82	2.90	7.50
4	IPAH47	59	1.63	80	290	1.86	468	93.7	57	71	2.37	12.95
5	IPAH48	55	1.72	72	523	1.85	500	94.2	21	70	2.90	4.00

H: height; W: weight; EO: endogenous ouabain; pM: picomolar; BSA: body surface area; 6-MWT: 6-minute walking test; SO_2: oxygen saturation; PAPm: mean pulmonary arterial pressure; CF: cardiac frequency; CI: cardiac index; PVRtot: total pulmonary vascular resistance (WU wood units are preferred to dynes.s.cm-5).

the 4th World Health Organization (WHO) symposium (2008), held in Dana Point, California, PAH can be idiopathic (IPAH) caused by unknown reasons, heritable/familiar or associated with other medical conditions such as connective tissue disease, HIV infection, portal hypertension (liver disease), sickle cell disease and congenital heart disease. Therefore, IPAH is a rare disease characterized by a progressive increase in pulmonary vascular resistance that leads to right heart failure. The right ventricle (RV) inability to adapt to post-load increasing is a crucial prognostic factor characterized by an increase in RV size, a decrease in systolic function, and consequent hypoxia increasing over time [12]. In adulthood, IPAH is more frequent in females than in males (1.6:1), probably due to hormones [13] and (or) genetic and autoimmune mechanisms [14]. However, we enrolled male patients as they were the only subjects available for the study at the time of data collection.

This study aims to investigate whether EO is detectable in the plasma of IPAH patients affected by chronic hypoxia.

METHODS

This study was approved by the Ethics Committee of Sapienza University (Policlinico Umberto I) in Rome, Italy (Ref. No. 4468; Protocol No. 303/17) and adhered to the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines. It was conducted in compliance with the protocol, data protection regulations, and all other regulatory requirements, as appropriate (STROBE). Each participant provided a written informed consent form before enrolling in the study.

Five Caucasian males were involved in this study. For each subject, we collected age, height, weight, EO plasma concentration, and specific variables to evaluate the severity of IPAH pathology *(Table 1)*. Blood sample collection for these patients was performed during their routine health monitoring. IPAH patients were selected from the Clinic database of the Pulmonary Hypertension Center of Policlinico Umberto I, Rome, Italy. Regarding the control subjects, we considered the EO concentrations of twenty-six (26) healthy subjects, which matched IPAH patients by gender, age, and ethnicity without any experience of breath-holding studied in Manfrini, et al. [7].

Blood sampling was carried out between 09:00 a.m. and 12:00 p.m. by collecting 4-6 mL of venous blood into EDTA collection tubes. Plasma was separated by centrifugation at 3,000 rpm for 15 minutes at room temperature and stored in a cryovial at -80 °C until analysis. Plasma EO is quantified by a Scintillation Proximity Assay (SPA) using Yttrium Silicate (YSi) beads (Perkin Elmer, Hebron, KY, USA) conjugated with a secondary antibody. These beads contain an embedded scintillant that emits light when bound with EO tritiated through the primary antibody. They are counted in a β-counter. The CPM (counts per minute) readings of the samples are translated, by software, into EO values. Please refers to Manfrini, *et al*. [7] for all details of this analysis.

Statistical analysis was performed using R version 3.2.1 (www.r-project.org) [15] and code programmed to specific studies and methodological approaches. Descriptive data were reported in jitter plots that show the median (thick line), the limits of the 95% confidence interval (thin line), the minimum and maximum values (short horizontal lines), and each value (small spots).

Data were studied using bivariate analysis to look for linear correlations between EO concentrations and the specific variables of IPAH pathology. The degree of correlation was assessed by the coefficient per rank of Spearman (ρs) that can take values between -1 (negative correlation) and +1 (positive correlation), 0 (no linear correlation). The Spearman coefficient has greater robustness than the Pearson coefficient for any outliers and deviations from the normality of the data.

Comparisons of endogenous ouabain concentrations between groups were performed by using the analysis of covariance (ANCOVA) to correct the analysis for confounding biometric data according to the linear correlation analysis previously performed. *Post-hoc* analysis was corrected by the Bonferroni method to contain type I error.

RESULTS

The plasma EO concentrations and specific markers of pathology severity are shown in *Table 1*. We ran a preliminary and exploratory correlation analysis among EO plasma concentrations and the markers collected. We found that EO concentrations were negatively correlated with the mean pulmonary arterial pressure (PAPm)

(ρs=-1, p<0.05) and the total pulmonary vascular resistance (PVRtot) (ρs=-1, p<0.05). Also, a positive correlation trend was observed with the cardiac index (CI) (ρs=0.82, p=0.09). These results are consistent with those found in Manfrini *et al*. [7], where comparisons of plasma EO concentrations between controls and diver groups showed a statistically significant category effect (p=0.034). In this study, plasma EO concentrations were significantly higher in IPAH patients than in the same control subjects. This comparison showed a statistically significant category effect ($p<10^{-6}$) *(Figure 1)*.

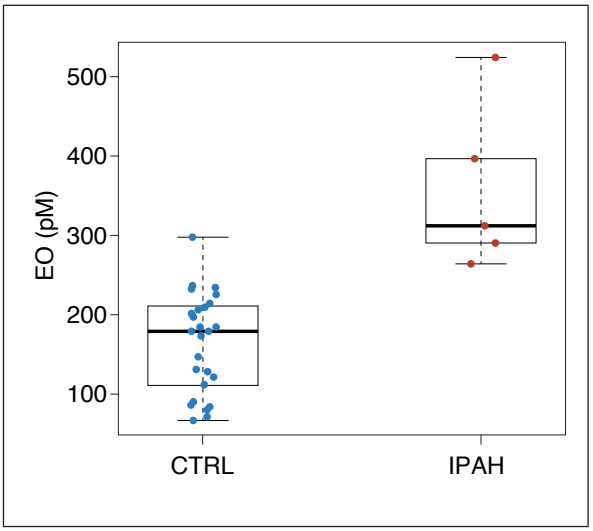

Figure 1
Jitter plots of plasma EO concentrations in 26 control subjects (CTRL) studied in Manfrini and colleagues [7] and 5 IPAH patients. EO is significantly higher ($p<10^{-6}$) in IPAH patients as compared to controls. Bars represent means ± standard deviation (SD); EO: endogenous ouabain; pM: picomolar; IPAH: idiophatic pulmonary arterial hypertension.

DISCUSSION

This study is preliminary, so we first measured EO in a peculiar hypoxia pathologic human model. IPAH patients are good models as they are exposed to chronic low oxygen levels [12]. We observed that IPAH patients had higher EO values than the control subjects without any breath-holding experience (no divers, no other sports activity involving apnea). This result is in line with data showing that EO plasma concentrations are higher in some clinical conditions that often occur in comorbidity with IPAH, like heart failure [16] and acute kidney injury [17]. It is known that the ouabain inhibits the activity of the Na+/K+-ATPase pump. The cell energy consumption by this enzymatic system is high [18, 19]. In these patients, the activity reduction of the pump might be a compensatory strategy to reduce cell energy consumption to adapt the ion balance and increase the resistance to low oxygen levels.

Although the number of IPAH patients was small, we explored correlations between EO and some specific clinical markers of disease severity. The limited sample size and the lack of long-term monitoring of the EO plasma concentrations do not allow reaching conclusions and make the analysis not very powerful. However, the choice to carry out this analysis, in any case, is motivated by the essentially descriptive and preliminary nature of the study in which even slight trends can be suggestive of hypotheses of interest. We found that EO plasma concentrations negatively correlated to the average pulmonary arterial pressure and total pulmonary vascular resistance and positively correlated (trend) to the cardiac index, a marker of healthy cardiac output. These findings, even though preliminary, could suggest that high concentrations of EO are predictive of a better adaptation of the right ventricular afterload.

CONCLUSIONS

These results, if confirmed with a larger sample, could have an impact on clinical practice as higher EO plasma concentrations might represent a positive prognostic index in IPAH patients and a helpful hint for future investigations involving other pathological conditions, for example, obstructive sleep apnea for possible therapeutic and diagnostic approaches.

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Authors' contributions

VM performed the experiment, analyzed the data, and wrote the manuscript; RB conceived the idea of the study and contributed to the study design; EM measured EO concentration in all samples and helped write the manuscript; RP supervised IPAH patient recruitment; RT executed IPAH patient recruitment and blood sampling; PM and CDV supervised the whole study and revised the manuscript. All Authors approved the manuscript and the version to be published.

At the time of data collection and analysis, Valerio Manfrini's affiliation was the Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Rome, Italy.

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Conflicts of interest statement

The Authors declare no conflict of interest.

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